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EPITHELIAL WATER PERMEABILITY IN THE EURYHALINE MUSSEL *GEUKENSIA DEMISSA:* DECREASE IN RESPONSE TO HYPOOSMOTIC MEDIA AND HORMONAL MODULATION[1]

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Abstract

The diffusional water permeability of isolated mantles from the mussel *Geukensia demissa* was reduced by incubation of the tissues in hypoosmotic media. The permeability of mantles from 1000 mOsm seawater (SW)-acclimated animals was 6×10^{-5} cm/s. A four-hour incubation in 500 mOsm SW or 250 mOsm SW reduced the water permeability by 2×10^{-5} cm/s and 4×10^{-5} cm/s, respectively. A half-hour exposure to the hypoosmotic medium was sufficient to induce the decrease in permeability.

The water permeability of mantles incubated in isosmotic SW containing acetone extracts of ganglia from 1000 mOsm SW-acclimated mussels or of mantle from 500 mOsm SW-acclimated mussels was significantly reduced. Extracts of gill had no effect.

Ovine prolactin (50 mg/ml) decreased the water permeability of mantles in isosmotic seawater. Cortisol (10^{-4} *M*), arginine vasopressin (10^{-6} *M*), and the molluscan neuropeptide FMRFamide (10^{-6} *M*) had no effect.

These results show that the epithelial water permeability of euryhaline bivalves varies with changes in the ambient salinity, and that these permeability changes may be modulated by factors of neural origin.

Introduction

A number of specific physiological mechanisms facilitate the survival of euryhaline marine animals in habitats characterized by variations in salinity. These mechanisms include changes in urinary output, regulation of the extracellular fluid composition, volume regulation, and changes in epithelial permeability to water and ions.

A reduced epithelial permeability to water in dilute media has been reported in several invertebrates, including a number of arthropods (Rudy, 1967; Smith, 1970a; Capen, 1972; Smith and Rudy, 1972; Cornell, 1973; Hannen and Evans, 1973; Lockwood *et al.,* 1973; Roseijadi *et al.,* 1976; Thuet, 1978), three polychaetes (Smith, 1964, 1970b; Fletcher, 1974), and a bivalve (Prusch and Hall, 1978). The phenomenon has been observed in osmoconformers (*e.g., Mytilus edulis, Libinia emarginata*) and in osmoregulators (*e.g., Rhithropanopeus harrisi, Nereis limnicola*). Nearly all of these data were collected by measuring changes in the fluxes of water into or out of whole animals exposed to dilute media. As indicated by Cornell (1979), part of the observed decreases in the flux of water across the epithelium of an intact animal could be effected by changes in circulation or ventilation. This criticism does not apply to

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studies of the response of the epithelial permeability of isolated tissues to changes in salinity.

Isolated tissues from only a few marine invertebrates have been used to examine changes in water permeability in response to dilution of the medium. Cantelmo (1977) found that the water permeability of gut epithelia and gills from the crabs *Cancer irroratus* and *Callinectes sapidus* was lower in tissues isolated from animals acclimated to 40% seawater than in tissues from animals acclimated to 100% seawater. However, exposure of isolated tissues to hypoosmotic media for 2 h did not change their water permeability. Acclimation of the mussel *Mytilus edulis* to reduced salinity caused a decrease in the diffusional permeability of water across isolated mantle tissues (Prusch and Hall, 1978).

Neurohormones have been implicated in the modulation of water permeability in a variety of invertebrates. For example, extracts of the thoracic ganglion decrease the water flux across isolated crab gut and gills (Mantel, 1968; Berlind and Kamemoto, 1977), and the injection of a brain homogenate reduces the rate of water exchange of earthworms (Carely, 1981). Two lines of evidence suggest a possible modulation of water balance by neural products in gastropod molluscs. Injection of synthetic thyrotropin releasing hormone into the freshwater snail *Lymnaea stagnalis* causes a slight loss in wet weight (Grimm-Jorgenson, 1979). Similarly, injection of homogenized R-15 cells from the abdominal ganglion of the opisthobranch *Aplysia brasiliensis* resulted in a 5% gain in wet weight (Kupfermann and Weiss, 1976).

The present study was undertaken to determine the response of the epithelial water permeability of isolated mantle tissue from the euryhaline mussel *Geukensia demissa* to decreases in the ambient osmotic concentration. This tissue was also used as a bioassay system to determine the effects of extracts of the ganglia and other tissues on water permeability.

The results show that the diffusional water permeability (P_d) of the mussel decreases in response to the dilution of the external medium, and that water permeability may be modulated by a factor of neural origin.

Materials and Methods

Animals

Atlantic ribbed mussels, *Geukensia demissa granosissima,* were collected from a salt marsh near St. Augustine Beach, Florida. The animals were kept unfed in running seawater (30‰) at ambient temperature. All animals were used within 3 weeks of collection.

Histology

The adductor muscles of individual mussels were cut, the animals opened, and pieces of the central portion of the mantle dissected free. The tissue was fixed in filtered seawater containing 2% glutaraldehyde, dehydrated and cleared in a graded series of water/ethanol/t-butyl alcohol, and embedded in paraffin. Sections (10 μm) were cut on a microtome, stained with hematoxylin eosin, and mounted on glass slides.

Measurement of diffusional water permeability

The adductor muscles of individual mussels were cut and the valves carefully pried apart. Each mussel provided two tissues, the mantle covering the inside of the

right valve and the mantle covering the inside of the left valve. The left and right mantle halves were cut away from the visceral mass and detached from the margins of their respective valves. The isolated tissues were placed in small covered dishes containing 5 ml of 1000 mOsm seawater (SW) which was aerated via small bore tubing. After 60 minutes of incubation, one mantle half was mounted over the aperture of one half of a diffusion chamber and secured with a soft rubber o-ring. The tissues were always mounted in the chamber so that the movement of tritated water was from the extrapallial cavity side to the mantle cavity side. Five ml of medium were placed in the chamber to provide a hydrostatic pressure head sufficient to check the mounted tissue for obvious leaks. The chamber was then assembled and both sides filled with medium by alternate additions of 2–3 mls. The total volume of each compartment of the chamber was 14 ml; the area of exposed tissue was 1 cm^2. Mixing and aeration were provided by gas lift pumps powered by water-saturated air. About 1 μCi of tritiated water was added to one compartment of the chamber (the "hot" side), and following 10 minutes equilibration, 100 μl samples of the medium in the other compartment (cold side) were removed at 15 minute intervals for 60–90 minutes. These samples were mixed with 10 ml scintillation cocktail and counted on a liquid scintillation counter. In experiments using paired left and right mantles, the flux across one mantle was measured while the matching tissue was transferred to a dish containing either control (1000 mOsm SW) or experimental medium. These matching tissues were further incubated from one half to four hours and then their P_d values determined in the same rinsed and dried chamber used for the matching control measurement.

The water flux across the tissue and the diffusional water permeability were calculated from equations 1 and 2, respectively:

1. $$J_{12}^{H_2O} = \left(\frac{1}{t}\right)\frac{Q_2^*}{Q_1^*/Q_1}\left(\frac{1}{A}\right)$$

2. $$P_d = \frac{J_{12}^{H_2O}}{C_w}$$

Q_1^*/Q_1 = specific activity in compartment 1
Q_2^* = amount of isotope in compartment 2
t = time
A = area of tissue
C_w = molar concentration of water

The differences in P_d values between paired left and right mantles were averaged and differences among treatment means assessed by Student's *t* test.

Tissue extracts

The pedal, visceral, and cerebral ganglia from 250 mussels acclimated to 1000 mOsm SW were dissected from the animals and pooled in a large volume of cold acetone to extract putative hormones and inactivate proteolytic enzymes; acetone extracts were also made of the gills and mantles from these mussels. The mantles of 200 mussels acclimated to 500 mOsm SW were also extracted in acetone. The extracts were evaporated to dryness on a rotary evaporator and the water soluble portion of the residue taken up in a minimal volume of distilled water. The dose added to the incubation media was approximately 1 animal equivalent. All tissue extracts and hormones were added to the incubation media and to the fluid in both compartments of the diffusion chamber.

Results

Histology

Figure 1 shows a cross section of the central portion of the mantle. This complex tissue separates the extrapallial space from the mantle cavity. Both surfaces are lined

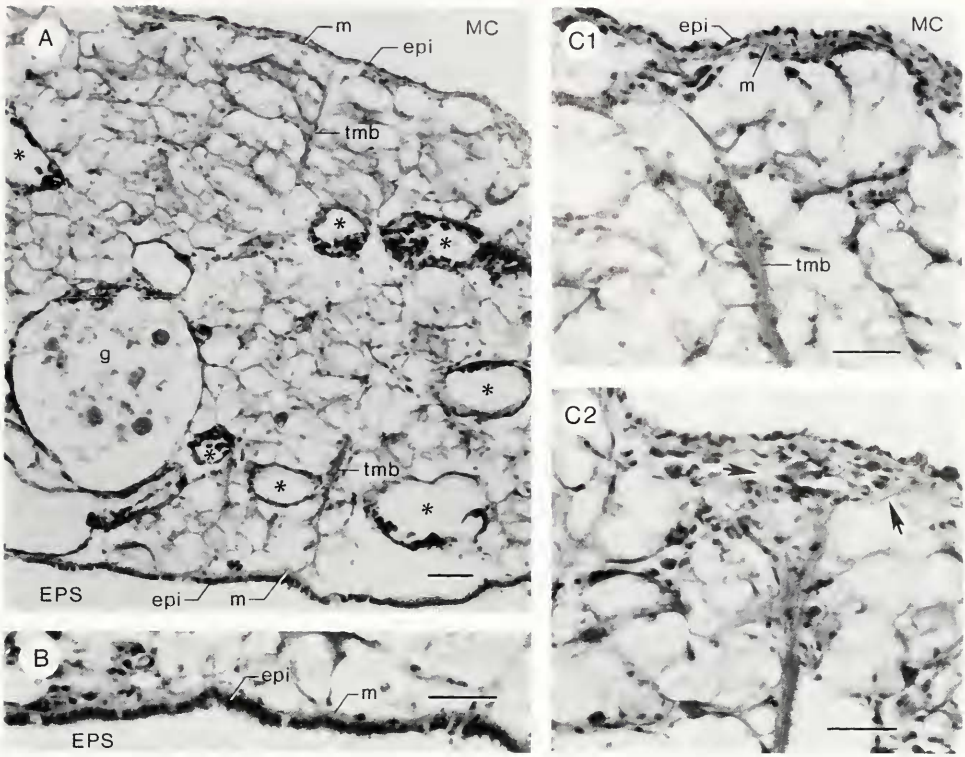

FIGURE 1. Cross-section through the central portion of the mantle of *Geukensia demissa*. A. Section from the mantle cavity (MC) to the extrapallial space (EPS) showing the epithelia (epi) on both surfaces with underlying muscle layers (m). The epithelia are separated by connective tissue which encompasses many hemolymph vessels (*), few genital follicles or canals (g), and transverse muscle bundles (tmb) connecting the two subepithelial muscle layers. Bar = 200 μm. B. Columnar extrapallial epithelium with thin underlying muscle layer. Bar = 100 μm. C. Squamous mantle cavity epithelium with thick underlying muscle layer. Fibers from the transverse muscle bundles splay out to join the subepithelial muscle layer (arrows in C2). Bar = 100 μm.

with an epithelium underlain by a muscle layer (Fig. 1A). The extrapallial epithelial cells are much taller than those lining the mantle cavity, but the subepithelial muscle layer associated with the extrapallial space is much thinner than that on the mantle cavity side of the tissue (compare Figs. 1A, $1C_1$). The bulk of the mantle is occupied by connective tissue in which are found numerous hemolymph vessels and, in these non-reproductive specimens, occasional genital canals (Fig. 1A). Bundles of muscle fibers traverse the mantle joining the two subepithelial muscle layers (Figs. 1A, $1C_2$). Similar structures have been described for the mantles of other species (Beedham, 1958).

Diffusional water permeability

Preliminary experiments showed that the accumulation of counts in the cold compartment of the diffusion chamber was linear with time for over six hours, indicating that the 10 minute equilibration with labelled water was sufficient for attainment of a steady-state flux across the tissue. The diffusional water permeabilities of

TABLE I

Changes in diffusional water permeability (P_d) of mantles from 1000 mOsm seawater-acclimated Geukensia demissa *after a four hour incubation in various seawaters*

Treatment medium	1000 mOsm	500 mOsm	250 mOsm
Change in P_d	0.5×10^{-5}	***-2.3×10^{-5}	***-4.1×10^{-5}
SD	1.1×10^{-5}	3.0×10^{-5}	1.3×10^{-5}
n	18	9	7

Values are in cm/s and represent differences in P_d between paired left and right mantles: one mantle of each pair was incubated in 1000 mOsm SW for 1 h; the other was incubated in the treatment medium for 4 h. Values significantly different from the 1000 mOsm treatment are marked with *** ($P < .001$).

mantles from animals acclimated to 1000 and 500 mOsm for three weeks were, respectively, $7.9 \pm 3.3 \times 10^{-5}$ (n = 10) and $4.3 \pm 0.7 \times 10^{-5}$ cm/s). These values are higher than the mean (2.2×10^{-5} cm/s obtained by Prusch and Hall (1978) for mantles of *G. demissa* acclimated to 1000 mOsm SW. Their animals, collected near Woods Hole, Massachusetts, were undoubtedly the subspecies *G. demissa demissa.* Differences in chamber design and differences between the two *G. demissa* subspecies probably account for the discrepancy.

There were no significant differences between the mean P_d values of mantles incubated for four hours in 1000 mOsm SW ($6.1 \pm 2.9 \times 10^{-5}$ cm/s) and the mean P_ds of the matching control (incubated in 1000 mOsm SW for 1 h) tissues ($6.5 \pm 2.9 \times 10^{-5}$ cm/s), nor was the mean of the differences between paired tissues ($0.5 \pm 1.1 \times 10^{-5}$ cm/s) significantly different from zero. In contrast, the water permeability of mantles incubated in 500 or 250 mOsm SW for four hours was decreased by ⅓ and ⅔, respectively, compared to paired controls (Table I). The data from a representative experiment are shown in Figure 2. The movement of labelled water across both tissues is linear with time; the movement of water across the tissue incubated in 500 mOsm SW is slower. The magnitude of the reduction in flux is constant throughout the experiment.

The time course of the reduction of epithelial water permeability (P_d) in 500 mOsm SW is summarized in Table II. A thirty minute incubation in dilute seawater was sufficient to induce a decrease of about 1×10^{-5} cm/s in the P_d value. Longer incubations further reduce the permeability, but these values were not significantly different from that induced by a thirty minute incubation (Table II).

When mantles were incubated in isosmotic medium containing an extract of ganglia from 1000 mOsm SW-acclimated mussels or an extract of mantles from 500 mOsm SW-acclimated mussels, the water permeabilities were significantly reduced. Extracts of other tissues had no effect (Table III).

Hormones which affect the water permeability of vertebrate tissues were tested for effects on the bivalve mantle. Ovine prolactin significantly reduced the P_d value of mantle tissues in isosmotic media. Neither arginine vasopressin nor cortisol changed the permeability of mantles in isosmotic SW (Table IV). The small reduction in the P_d value of tissues incubated in isosmotic media with the molluscan neuropeptide FMRFamide was not significant (Table IV). Colchicine did not prevent the decrease in P_d induced by exposure to dilute media (Table V).

DISCUSSION

The diffusional water permeability (P_d) of isolated mantles from the euryhaline mussel *Geukensia demissa* decreases when the tissues are exposed to hypoosmotic

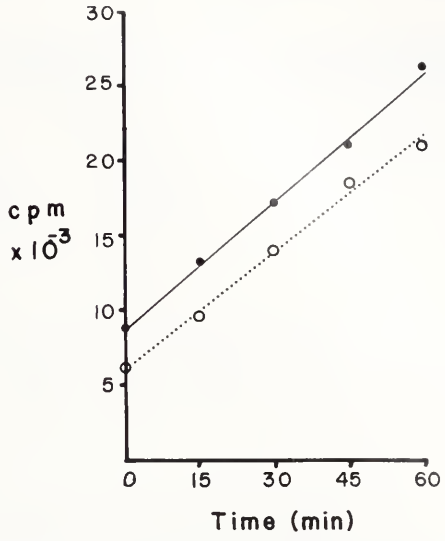

FIGURE 2. The unidirectional movement of tritiated water across a piece of isolated mantle of *Geukensia demissa*. Total counts per minute appearing in the "cold" side of a diffusion chamber are plotted as a function of time. The data are from paired mantle tissues from one mussel: the left mantle was incubated for 1 h in 1000 mOsm seawater (solid circles); the right mantle was incubated in 500 mOsm seawater (open circles) prior to measurement of the tritiated water flux in a diffusion chamber containing the same media.

media; the decrease in permeability is proportional to the magnitude of the decrease in the ambient osmotic concentration. Furthermore, the P_d of the isolated tissue incubated in isosmotic medium is reduced by a vertebrate hormone and by an endogenous factor of neural origin.

The reduction in permeability induced by a 30 min exposure to 500 mOsm seawater is less than that resulting from long-term acclimation of the mussels to 500 mOsm SW. The reduction of water permeability by ganglion extracts from 1000 mOsm-SW acclimated mussels and mantle extracts from 500 mOsm-SW acclimated mussels suggests that the putative factor is produced in the ganglia and released to the periph-

TABLE II

Time course of change in water permeability (P_d) of mantles from 1000 mOsm seawater-acclimated Geukensia demissa *during incubation in 1000 mOsm or 500 mOsm seawater*

	Treatment medium									
	1000 mOsm					500 mOsm				
Incubation duration (h)	0.5	1	2	3	4	0.5	1	2	3	4
P_d change ($\times 10^{-5}$ cm/s)	0.9	0.4	0.3	0.4	0.2	*−0.9	***−1.0	**−1.4	***−1.9	***−1.9
SD	1.1	0.4	1.4	0.9	0.9	0.6	0.9	1.4	1.8	1.4
n	4	3	4	8	5	4	10	9	10	7

Values are differences in P_d between paired left and right mantles: one mantle was incubated in 1000 mOsm SW for 1 h; the other was incubated in the treatment medium for 0.5 to 4 h. Values marked with asterisks are significantly different from the corresponding 1000 mOsm treatment value (* = $P < .05$, ** = $P < .01$, *** = $P < .001$)

TABLE III

The effect of various tissue extracts on the water permeability (P_d) of mantles from 1000 mOsm seawater acclimated Geukensia demissa *incubated four hours in 1000 mOsm seawater*

	Treatment medium				
	1000 SW	1000 SW + 1000 mantle ext.	1000 SW + 1000 gill ext.	1000 SW + 1000 ganglia ext.	1000 SW + 500 mantle ext.
P_d change	0.5	1.3	0.6	*−1.4	**−1.5
SD	1.4	1.1	1.6	1.2	1.9
n	18	6	5	3	10

Values are $\times 10^{-5}$ cm/s and represent differences in P_d between paired left and right mantles: one mantle was incubated in 1000 mOsm SW for 1 h; the other was incubated in the treatment medium for 4 h. Values marked by asterisks are significantly different from that for 1000 SW (* = $P < .05$; ** = $P < .01$).

ery during acclimation to low ambient salinity. Prusch and Hall (1978) observed a 67% reduction in the water permeability of mantles isolated from the mussel *Mytilus edulis* during four weeks of acclimation to 70% seawater. Thus, in the intact animal, continuous release of neural factors may facilitate a larger decline in permeability than occurs in isolated tissues. As yet there are no data on the size, structure, or chemical nature of this putative neurohormone. However, it is not FMRFamide (Table III).

Prolactin reduced the water permeability of *G. demissa* mantles in isosmotic media (Table IV). Prolactin also reduces the permeability of teleost epithelia to water and ions (Doneen and Bern, 1974; Foskett *et al.*, 1983). While the presence of prolactin has been demonstrated by immunocytochemical methods in ascidians (Pestarino, 1984), it has not been reported in any other invertebrate. Therefore it is unlikely that the active substance in *G. demissa* ganglia is prolactin.

Khan and Salueddin (1979; 1981) associated changes in the anatomy of the septate junctions between kidney cells in the snail *Helisoma duryi* with increased water permeability. Extracts of the visceral ganglia induce these changes which occur within

TABLE IV

The effects of selected hormones on the change in diffusional water permeability (P_d) of mantles from 1000 mOsm seawater-acclimated Geukensia demissa *incubated four hours in 1000 mOsm seawater*

	Treatment medium				
	1000 SW	1000 SW + Prolactin (50 μg/ml)	1000 SW + FMRFamide (10^{-6} *M*)	1000 SW + cortisol (10^{-4} *M*)	1000 SW + Arg vasopressin (10^{-6} *M*)
P_d change	0.5	***−2.3	−0.4	−0.5	−0.4
SD	1.1	1.4	0.9	1.5	2.0
n	18	6	4	4	5

Values are $\times 10^{-5}$ cm/s and represent differences in P_d between paired left and right mantles: one mantle was incubated in 1000 mOsm SW for 1 h; the other was incubated in the treatment medium for 4 h. The value marked by *** is significantly different from that for 1000 SW ($P < .001$).

TABLE V

The effect of colchicine on the water permeability (P_d) of mantles from 1000 mOsm seawater-acclimated Geukensia demissa *incubated four hours in 500 mOsm seawater*

	Treatment medium	
	500 SW	500 SW + colchicine (2×10^{-4} *M*)
P_d change	−2.3	−2.0
SD	3.0	3.1
n	9	3

Values are $\times 10^{-5}$ cm/s and represent differences in P_d between paired left and right mantles: one mantle was incubated in 1000 mOsm SW for 1 h; the other was incubated in the treatment medium for 4 h.

30 minutes. Neurohormones, then, can alter the water permeability of molluscan epithelia by causing changes in the structure of the tissues, thereby changing the resistance of the paracellular pathway to water movement. The failure of colchicine to prevent a decrease in the P_d of mantles exposed to dilute media (Table V) suggests that microfilament activity is not involved in the process.

If the major route of water movement across the *G. demissa* mantle is paracellular, osmotic swelling of the epithelial cells could contribute to a decrease in the water permeability of the tissue during exposure to hypoosmotic media. Isolated *G. demissa* ventricles exposed to hypoosmotic seawater stop beating, but the mechanical activity of the ventricle recovers within 90–120 minutes (Pierce and Greenberg, 1972). Recovery of the mechanical activity of the ventricle apparently is due to cellular volume regulation. If the time course of recovery of cellular volume by the mantle cells is similar to that of the myocardial cells, osmotic swelling cannot account for the reduction in water permeability induced by 2–4 h incubations in hypoosmotic seawater. However, in the absence of data on the time course of changes in the volume of mantle cells exposed to hypoosmotic stress, the possibility that cell swelling accounts for some or all of the decrease in epithelial permeability cannot be ruled out.

The mantle of *G. demissa* is vascularized (Fig. 1) and therefore well-perfused by the circulation. Mounting the tissues in the diffusion chamber precluded perfusion, and therefore the effects of delivery of the tissue extracts and other drugs via the circulation cannot be assessed.

Extracts of various nervous tissues affect epithelial water permeability of crustaceans and annelids (Mantel, 1968; Tullis and Kamemoto, 1974; Berlind and Kamemoto, 1977; Carely, 1981). While none of these factors has yet been identified, it is clear that neural factors modulate water permeability in euryhaline invertebrates.

In summary, the epithelial water permeability of euryhaline molluscs changes during acclimation to changes in the ambient salinity. These changes in permeability may be modulated by one or more neural factors of unknown structure. The mechanisms responsible for increases or decreases in water permeability apparently involve changes in the junctional complexes between the epithelial cells, but factors affecting transcellular water permeability, such as the insertion or removal of water channels or changes in the composition of the membrane lipid bilayer, cannot be ruled out.

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